PATHOGENICITY OF *PRATYLENCHUS BRACHYURUS* TO CITRUS

By

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INTRODUCTION

Species of the nematode genus Pratylenchus are found throughout the world and rank among the most important of the pests causing root destruction of plants (15). Routine samples of soil and roots from declining and healthy citrus obtained from the important citrus producing areas during the past 10-12 years indicate that species of Pratylenchus are widely distributed in the citrus groves of Florida. According to Christie (3), this may be the plant parasitic nematode genus that occurs most frequently in Florida citrus groves. A morphological study of the Pratylenchus species that is associated with the roots of citrus in Florida (2) indicated that this species falls within the description of Pratylenchus brachyurus (Godfrey, 1929) Filipjev and Stekhoven, 1941, as given by Sher and Allen (15).

Pratylenchus brachyurus is taxonomically related to the burrowing nematode, Radopholus similis (Cobb, 1893) Thorne, 1949, the causal agent of spreading decline of citrus in Florida (17). Since P. brachyurus is of common occurrence on citrus trees, the possibility exists that it may be causing significant damage to citrus roots.

Consequently, information on its morphology, life cycle and feeding habits would aid in a decision as to its actual pathogenicity.

Experiments were initiated by the author in 1961 to determine the extent of damage *P. brachyurus* might cause to citrus and to compare this damage with that caused by *R. similis*. It was also hoped that the investigation would provide information as to how much injury a nematode can cause to a citrus root before the damage is fatal to the root involved.

LITERATURE REVIEW

In 1880, deMan described and named the lesion nematode, *Tylenchus pratensis*, from the Netherlands and England. Scribner (13) reported a disease of potato tubers incited by the lesion nematode that subsequently was named *Pratylenchus scribneri* by Steiner in 1943. Zimmermann (21) discovered a nematode (*Tylenchus coffeae*) severely infecting coffee roots in Java. Cobb (4) described *Tylenchus penetrans*, and reported it as attacking potato tubers and the roots of cotton, camphor and violets. Godfrey (8) described the lesion nematode, *Tylenchus brachyurus*, in 1929. He designated it as the incitant of a destructive disease of pineapple and other plants in Hawaii. His description was based on females and no males were found. Filipjev (7) erected the genus *Pratylenchus* in 1934 to include the lesion nematodes discussed above.

Steiner (16), in 1949, reported a nematode infection of potato from Florida. He described the parasite as a species belonging to the genus *Pratylenchus* and gave it the name *Pratylenchus leiocephalus*. Once again males were not found. Sher and Allen (15) synomized *P. leiocephalus* to

P. brachyurus and described the male for the first time, on the basis of two individuals.

Lordello, Zamith and Bouck in 1954 described a lesion nematode from potatoes in Brazil, and named it *P. steineri* (11). Then Lordello, Zamith and De Arrudas (12) described the same parasite from cotton and soybeans. Again the description was based upon females only. Later, *P. steineri* was placed in synonymy with *P. brachyurus* (10).

Christie (3) states that the burrowing nematode is essentially a lesion nematode and the character of the injury it causes to roots is much the same as that caused by lesion nematodes of the genus *Pratylenchus*. After entering a root, the nematodes feed upon and destroy the cells of the cortical region and bring about small necrotic lesions or cavities. Here the females reach maturity and reproduce. Through the feeding of a steadily increasing number of parasites, a necrotic lesion is enlarged and may join adjacent lesions until large areas are affected.

DuCharme (5) reported that *R. similis* entered actively growing tips of citrus roots and that all types of parenchyma cells were attacked. The nematodes seemed to prefer the phloemcambium ring which was virtually destroyed. Hyperplasis and tumor formation occurred when the nematodes became situated next to the pericycle. As the nematodes progressed through the root, tunnels and cavities were formed in the cortex and stele.

Feder and Feldmesser (6) reported that citrus seedlings infected by R. similis were badly stunted and chlorotic, that the reduced numbers of roots were covered with lesions, and that the roots were distorted in shape. Root tips were swollen, spatulate or "football-shaped"; large elongate or round swellings were present along the entire lengths of some roots, giving them a beaded appearance. Some lesions coalesced to form large cankers with callused margins. Cankers had dark centers and firm brown margins. Upon dissection, lesions and swollen areas alike yielded large numbers of all life cycle stages of R. similis.

Godfrey (8) observed that lesions on pineapple roots caused by P. brachyurus began as elongate brown spots which became darker with age. Borders of the lesions were sharply delineated by dead, discolored epidermal cells from which the affected tissues extended inward to the stele. Coalescing lesions eventually involved the entire cortical region; then the vascular tissues died, and the root was destroyed. Variations in susceptibility were evident in several hosts. Roots of cowpea suffered pronounced damage, while those of tomato and sugar cane were affected but little.

Prior to the initiation of the experiments reported herein, nothing was known of the effects *P. brachyurus* might have on citrus. In fact no major work had been completed pertaining to the effects of the feeding by this species of *Pratylenchus* on the roots of plants.

MATERIALS AND METHODS

Colonies of *Pratylenchus brachyurus* and *Radopholus similis* were obtained when needed from naturally infected citrus trees. Fresh collections were made for each experiment by extracting samples of feeder roots at soil depths ranging from 10 to 48 inches. Individual samples were placed in glass jars and stored in insulated boxes containing ice in order to prevent overheating while being transported to the laboratory where samples were then processed immediately. In this manner it was possible to process all samples within 2-3 hours after they were collected.

Approximately 10 grams of the roots were washed thoroughly, then replaced in jars while wet and incubated for about 48 hours, following Young's (19) procedure. During this incubation period endoparasitic nematodes, such as members of the genera *Pratylenchus* and *Radopholus*, emerge from within the roots and specimens are collected in the free water surrounding the roots and the bottoms of the jars.

Following the 48-hour period, each container with its individual root samples was partially filled with water and shaken to remove the nematodes from the roots. The water containing the nematodes was then decanted onto a standard soil sieve containing 100 meshes per inch placed over a 325-mesh soil sieve. The material collected on the 325-mesh sieve contained the nematodes. This material was then removed with a small amount of water to Syracuse watch glasses and examined with the aid of a dissecting microscope and a compound microscope.

Identification of the *Pratylenchus* Species Associated with Citrus

Some specimens of the *Pratylenchus* in question collected by the above procedure were killed with gentle heat and fixed in a 5 per cent formalin solution. Some of these were examined after mounting in 5 per cent formalin on microscope slides. Others were processed, using Seinhorst's method (14), to dehydrated glycerine by transferring the specimens from the formalin to a Bureau of Plant Industry type watch glass half full of 1.5 per cent glycerine in 10 per cent ethyl alcohol. The watch glass was then placed in a preparation dish containing an excessive amount of 95 per cent ethyl alcohol. This was stored at approximately 38°C for about four hours. At this time, 5 per cent glycerine in 95 per cent ethyl alcohol was added to the specimens in the watch glass so as to double the volume of liquid. Most of the water and alcohol were removed by storing overnight at 38°C. The watch glass containing the specimens was then kept for at least two days in a desiccator containing calcium chloride, after which the specimens were mounted in dehydrated glycerine as outlined by Seinhorst (14). Other specimens were processed to glycerine by transferring the formalin-fixed specimens to lactophenol in a hollow cavity slide and by warming to about 65°C. From lactophenol, the nematodes were passed through each of five solutions containing different concentrations of glycerine, distilled water, lactic acid, phenol and formol. In this manner the nematodes were placed in pure glycerine in about one hour according to the procedure outlined by Baker (1). The latter two methods provided nematode specimens for the preparation of permanent microscope mounts using Cobb's aluminum slides. In all instances, the morphology of the nematode was studied with the aid of a research microscope equipped with an oil immersion objective. Camera lucida illustrations were prepared following the technique outlined by Thorne (18).

Pathogenicity of *Pratylenchus brachyurus* to Citrus

The nematodes obtained from citrus roots as described above were used to set up a test designed to establish pathogenicity of *P. brachyurus* on citrus roots. *R. similis* obtained by the same procedure were used for comparison. Mature females were individually hand-picked into distilled water and used to inoculate sour orange (*Citrus aurantium* L.) seedlings one inch in height, each growing in steam sterilized soil within 6-inch clay pots. Two hundred *P. brachyurus* were used to inoculate each of five seedlings. Two hundred *R. similis* were used to inoculate each of five additional seedlings. Five similar plants without nematodes were used as checks. The seedlings were kept in a greenhouse at temperatures ranging from 25-33°C. The plants were watered when necessary and fertilized according to established procedures.

Histopathology of Citrus Roots Attacked by *P. brachyurus*

Internal injury to roots by *P. brachyurus* and *R. similis* were studied after being processed by standard histological methods. Infected and non-infected roots were killed and fixed in Randolph's Modified Navashin fluid, dehydrated by a tertiary butyl alcohol method and embedded in Fisher's "Tissuemat." Sections were cut on a rotary microtome 10 microns thick, fixed to slides with Haupt's adhesive, stained with Johansen Quadruple stain and mounted in Fisher's "Permount." The procedure outlined by Johansen (9) was generally followed.

The finished slides were then studied with the aid of a research microscope. Photomicrographs were prepared of selected but representative sections.

Control of *P. brachyurus* and *R. similis* on Citrus

In an earlier experiment (2) pots containing citrus seedlings inoculated with *P. brachyurus* were moved from a greenhouse in Lake Alfred, Florida to a greenhouse on the campus of the University of Florida at Gainesville. Shortly after the transfer to Gainesville, the plants grew erratically and developed severe iron deficiency symptoms. An examination of samples obtained from the inoculated pots revealed that the populations of *P. brachyurus* were decreasing.

Before the plants were moved from Lake Alfred, the pots containing the plants and nematodes were watered with water from a deep well that contained no additives. The city water used in Gainesville had been treated with chlorine. Since some of the most effective nematocides known contain chlorine in the chemical structure, it seemed possible that concentrations of this halogen built up levels that were toxic to the nematodes and to the seedlings.

Therefore, calcium hypochlorite, a water soluble compound was used as a source of chlorine to treat citrus seedlings growing in soil naturally infested by nematodes in a greenhouse at Lake Alfred. Preliminary experiments indicated that *P. brachyurus* and *R. similis* could be killed by concentrations as low as five ppm chlorine while growing *in vitro*. Phytotoxicity tests on citrus seedlings indicated that citrus plants would withstand concentrations as high as 500 ppm for three months. Consequently, each of five sour orange seedlings were treated with a solution of calcium chlorite containing 500 ppm chlorine, five seedlings at 200 ppm, five seedlings at 100 ppm and five seedlings at 50 ppm. Five additional seedlings were treated with sodium hypochlorite solution at 100 ppm, and five seedlings treated with water only were used as checks. Three hundred cubic centimeters of the respective solutions were applied to each plant on each Monday, Wednesday and Friday for three months.

RESULTS AND DISCUSSION

Identification of the Pratylenchus Species Associated with Citrus

Studies of female specimens of the Pratylenchus species from citrus in Florida indicate that they are conspecific with Pratylenchus brachyurus as described by Sher and Allen (15). The dimensions of the females are:

L = 480-693u

a = 18.8-22.0u

b = 5.15-6.6u

c = 18.3-21.0u

V = 82.3-89.4 per cent

Stylet = 19-21u

Sher and Allen (15) apparently observed only two males, on which their description was based. During the present study some 40 males were found and examined. In general these appeared identical with the description by Sher and Allen (15); however, one important difference was observed. In describing the male, Sher and Allen (15) state "phasmid slightly posterior to middle of tail, do not extend into delicate bursa." In the more than 40 male specimens examined during the course of this study, the phasmids definitely extended into the bursa to form a small rib (Figs. 1 and 2). The following dimensions are given for these males:

L = 450-512u
a = 19.2-21u
b = 5.8-6.4u
c = 19.2-24u
T = 47-53 per cent
Stylet = 18-19u

The fact that the male bursal characteristic differs from the description of Sher and Allen (15) is interesting but the difference does not appear to warrant species separation. Also of interest is the apparent greater abundance of males in the citrus population. These males apparently do not mate since none of the many females examined during the course of this study contained a spermatheca and no sperm were observed within the uteri.

Pathogenicity of *Pratylenchus brachyurus* to Citrus

Citrus seedlings inoculated with *P. brachyurus* began to exhibit differences from check plants within three weeks and the differences increased with time. The inoculated plants were stunted, they possessed fewer leaves and on hot days wilting of the foliage was pronounced. After six

Fig. 1.-Female of Pratylenchus brachyurus; anterior portion of female; and lateral view of male tail showing phasmid extending into bursa to form a small rib.

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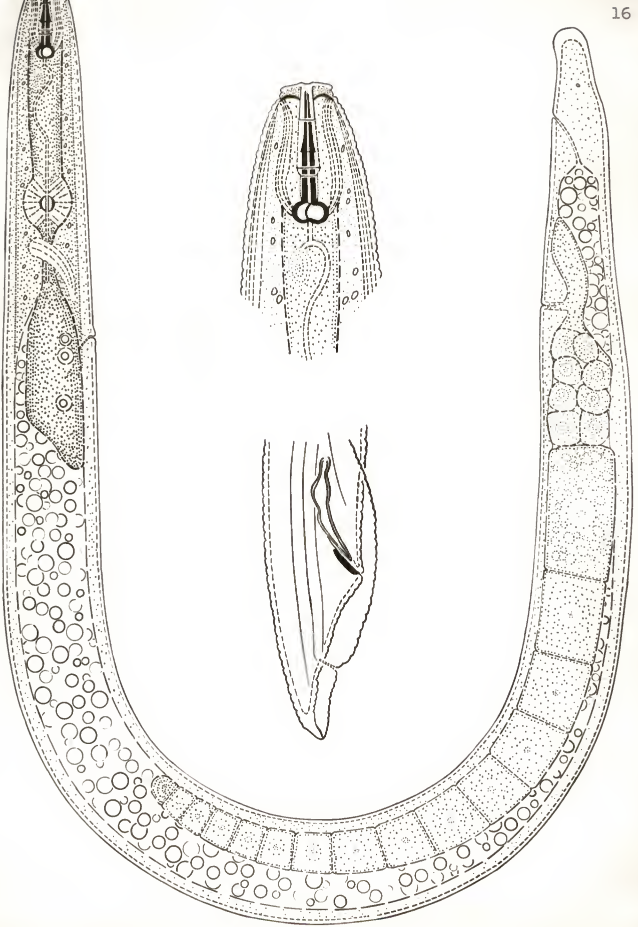
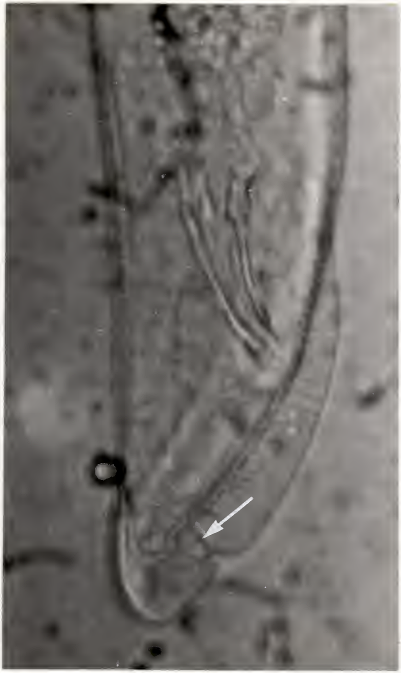


Fig. 2.-Photomicrograph of the male Pratylenchus brachyurus. Notice the phasmid extending into the bursa to form a small rib.



months, the root systems of infected plants were greatly reduced and distorted (Figs. 3 and 4) and brownish lesions were present on the feeder rootlets.

The plants that were initially inoculated with 200 mature *P. brachyurus* yielded an average of 404 specimens of all larval stages. Additional specimens were present but not counted in the sectioned roots. Statistical analysis of the data obtained from these experiments and the analysis of variance tables shows that differences in height, weight and number of leaves of the plants infected with *P. brachyurus* as compared with the check plants are statistically different at the 0.1 per cent level (Tables 1 and 2).

The plants that were inoculated with the burrowing nematode, *Radopholus similis*, also exhibited differences from the uninoculated plants and from those inoculated with *P. brachyurus*. Reduction in top growth was much more pronounced with *R. similis* than with *P. brachyurus* (Tables 1,2, and Fig. 4). The plants that were inoculated with 200 *R. similis* females yielded an average of 1141 specimens per plant. Thus, *R. similis* reproduced to a greater extent on citrus and brought about greater damage than did *P. brachyurus*.

Fig. 3.-Photograph of sour orange seedlings six months after inoculation with _R_. _similis_ (Rs) and _P_. _brachyurus_ (Pb). Note the pronounced reduction and distortion of roots from both _R_. _similis_ and _P_. _brachyurus_.

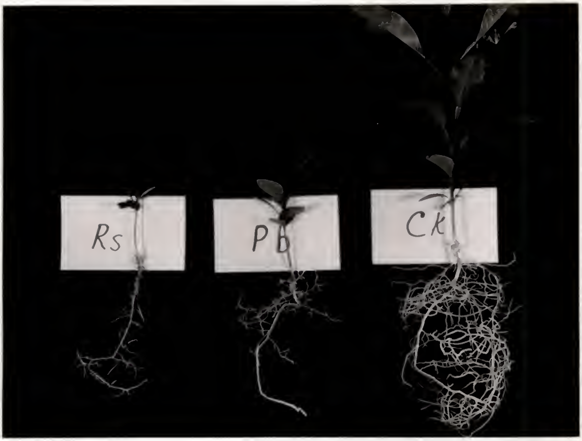

Rs
Pb
Ck

Fig. 4.-Photograph showing stunting of sour orange seedlings six months after inoculation with *R. similis* (Rs) and *P. brachyurus* (Pb).

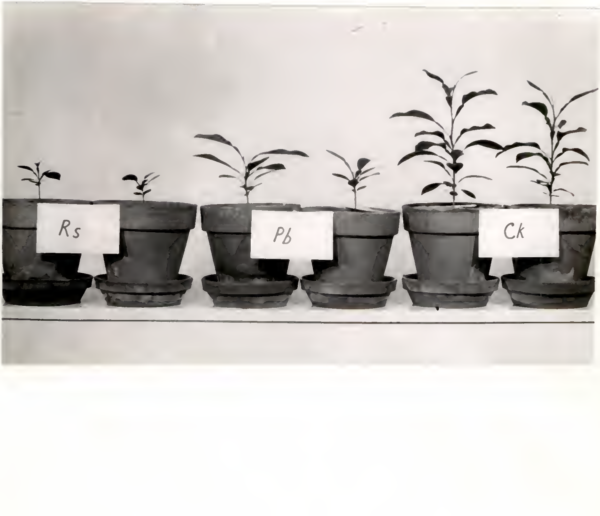

Rs
Pb
Ck

Table 1. Measurements of sour orange seedlings: (1) uninoculated, (2) inoculated with *P. brachyurus*, and (3) inoculated with *R. similis* grown in steam sterilized soil for six months.

		Height in inches	Number of leaves	Fresh weight total plant in grams	Fresh weight tops in grams	Fresh weight roots in grams
Uninoculated	1.	6.25	17	7.9	4.3	3.6
	2.	8.25	20	12.8	6.6	6.2
	3.	7.25	20	10.7	6.4	4.3
	4.	7.00	17	10.0	5.5	4.5
	5.	7.00	18	8.7	5.2	3.5
	Mean	7.15	18.4	10.02	5.60	4.42
Inoculated with *P. brachyurus*	1.	5.37	16	8.7	4.4	4.3
	2.	4.50	13	5.5	2.9	2.6
	3.	4.50	13	5.2	2.6	2.6
	4.	2.75	8	3.6	1.9	1.7
	5.	3.75	13	4.8	2.4	2.4
	Mean	4.17	12.6	5.56	2.84	2.72
Inoculated with *R. similis*	1.	1.50	5	.6	.3	.3
	2.	1.50	7	1.0	.5	.5
	3.	1.75	6	1.2	.5	.7
	4.	2.00	8	1.15	.35	.8
	5.	2.0	8	1.0	.5	.5
	Mean	1.75	6.8	.99	.43	.56

Table 2. Analysis of variances.

Source of variation	df	Sum of squares	Mean squares	F
Analysis of variance:	Height in inches (from data of Table 1)			
Treatments	2	73.10	36.55	88.072***
Error	12	6.23	.415	
Total	14	79.33		
		.01		
	R. similis	P. brachyurus	Checks	
	1.75	4.17	7.15	
Analysis of variance:	Number of leaves (from data of Table 1)			
Treatments	2	336.4	168.2	41.02***
Error	12	49.2	4.1	
Total	14	385.6		
		.01		
	R. similis	P. brachyurus	Checks	
	6.8	12.6	18.4	
Analysis of variance:	Total weight (from data of Table 1)			
Treatments	2	203.862	101.931	42.102***
Error	12	29.062	2.421	
Total	14	232.924		
		.01		
	R. similis	P. brachyurus	Checks	
	.99	5.56	10.02	

Table 2. Continued

Source of variation	df	Sum of squares	Mean squares	F
Analysis of variance: Weight of tops (from data of Table 1)				
Treatments	2	66.924	33.46	56.711***
Error	12	7.11	.59	
Total	14	74.034		

.01

R. similis	P. brachyurus	Checks
.43	2.84	5.60

Source of variation	df	Sum of squares	Mean squares	F
Analysis of variance: Weight of roots (from data of Table 1)				
Treatments	2	37.426	18.713	26.356***
Error	12	8.528	.71	
Total	14	45.954		

.01

R. similis	P. brachyurus	Checks
.56	2.72	4.42

***Significant at 0.1 per cent level.

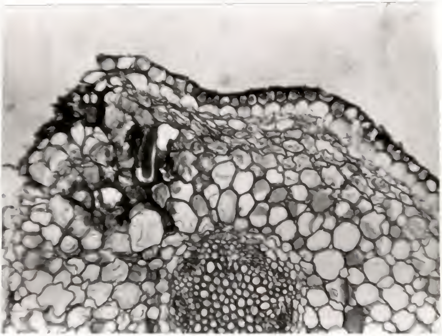
The results strongly indicate that the population of P. brachyurus used in the experiments will attack and reproduce upon at least one type of citrus. Furthermore, the data definitely indicate that this parasite is pathogenic to this citrus. Initially, it was hoped that this study would provide some insight into the reasons why R. similis appeared to be more pathogenic than P. brachyurus on citrus and thus provide some leads to explain the tremendous injury produced by R. similis on citrus. Since the two species reproduced at different rates, the different host reactions can be explained solely on the basis of population differences.

Histopathology of Citrus Roots Attacked by Pratylenchus brachyurus

Examinations of sections of citrus roots infected by P. brachyurus revealed that this parasite may bring about severe damage to the parenchyma cells of the cortical region (Figs. 5,6, and 7). In most instances, destruction of cortical parenchyma occurred, resulting in the formation of cavities or tunnels within the cortex. In some instances, the epidermis was ruptured or split; and in others, the epidermis appeared to be intact, indicating that the nematodes moved from one place to another inside the roots.

Fig. 5A.-Photomicrograph of a transverse section of a citrus root infected by P. brachyurus. Note portion of nematode body in cortical region.

Fig. 5B.-Photomicrograph of a transverse section of a citrus root infected by P. brachyurus. Note dark staining areas in the cortical region.



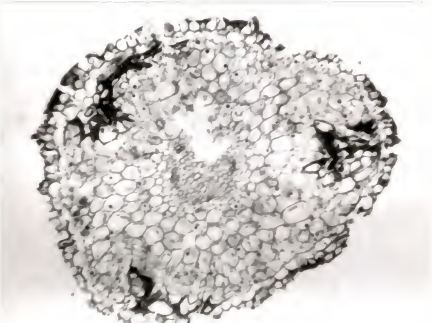


Fig. 6.-Photomicrograph of a longitudinal section of a citrus root infected by *P. brachyurus*.

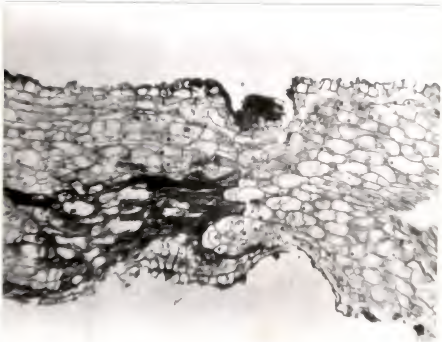
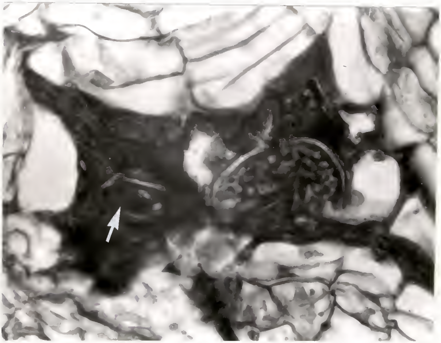


Fig. 7.-Photomicrograph of a transverse section of a citrus root showing a single P. brachyurus. Arrow is pointing to the nematode stylet.



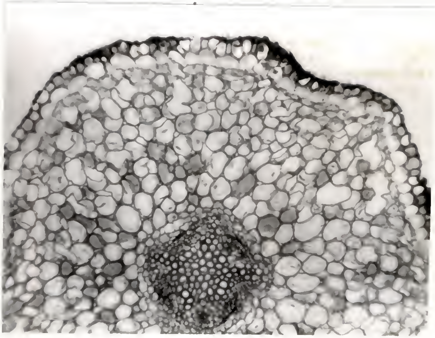
The walls of normal cells (Figs. 8 and 9) appeared green when stained as described, but those affected by the feeding activities of the nematodes were stained a bright red. Injury extended two to three cells on each side of the nematode. This indicated that injury to these cells was probably caused by the secretions of enzymatic toxins by the nematode. It appeared that the plant responded to these secretions by attempting to form a corky layer between the invaded cells and the healthy tissue. This is illustrated in Figure 5 which is a photomicrograph of a transverse section of an infected root showing breakdown in the cortical tissue.

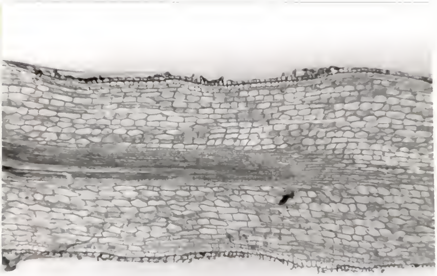
Damage to the vascular system of the plant was not observed. Only one to a few nematode specimens were observed in the various sections of roots that were invaded by P. brachyurus. This indicates, along with the numbers of specimens found in each plant on termination of the experiment, that this citrus plant species is not a good host from the standpoint of reproduction by the nematode.

Examinations of sectioned roots that were infected by R. similis show the presence of many individual nematodes within the root (Figs. 10,11, and 12). The cortical region and the pericycle were invaded by this nematode. Apparently, secretions by R. similis overcome

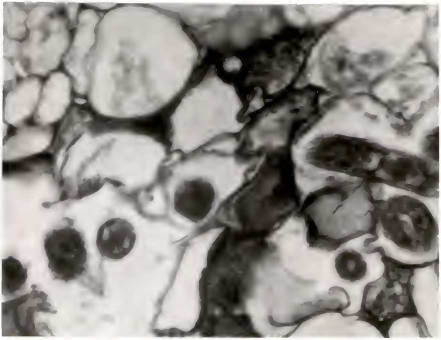
Fig. 8.-Photomicrograph of transverse section of a healthy root.

Fig. 9.-Photomicrograph of longitudinal section of a healthy root.





Figs. 10 and 11.-Transverse sections of a citrus root infected by R. *similis*. Note the numbers of nematodes present and the large cavities or tunnels.



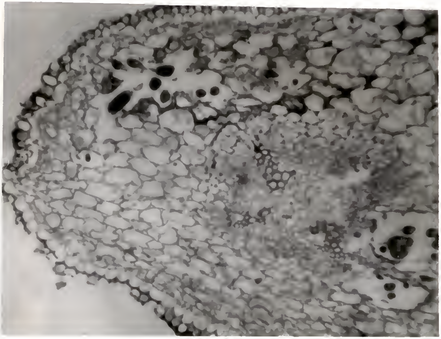
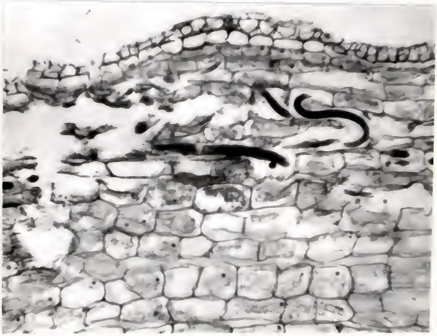


Fig. 12.-Photomicrograph of a longitudinal section of a citrus root infected by *R. similis*. Note the large cavities caused by the feeding of this nematode.



any plant antagonism to the presence of this nematode and thus damage is severe. Figures 10 and 11 show transverse sections of citrus roots infected by *R. similis* with numerous individuals within the cavities or tunnels created by the activities of this nematode. Figure 12 shows a longitudinal section of an infected root containing large cavities caused by this nematode.

The damage caused by these two nematodes appears to be similar; however, *R. similis* was observed feeding near the phloemcambium ring and *P. brachyurus* seemed not to disturb this area of the root. Histological sections of the roots also showed that *R. similis* was more numerous inside the root tissue than *P. brachyurus*. Feeding secretions of the nematodes probably caused more damage through host response than through the mechanical damage which was caused by the entering and moving about of the nematode inside the root. It would be interesting to determine the exact nature of the secretions by both nematodes for comparison.

In no case has field damage from *P. brachyurus* been observed to approach that of the spreading decline disease incited by *R. similis*. This is true even when rather large populations of *P. brachyurus* are found. There appears to be a decided difference in host response to the feeding activities of *P. brachyurus* and *R. similis*. In each replication, *R. similis* reduced both top and root growth to a significantly greater extent than *P. brachyurus*. A possible explanation could be that, under the conditions of these experiments, conditions were more conducive to the feeding and reproduction of *R. similis* than *P. brachyurus*. Another possible explanation is that citrus is a more suitable host of *R. similis* than *P. brachyurus*. In any event, the feeding of *R. similis* sharply reduced the growth of the citrus plants almost immediately.

Control of *P. brachyurus* and *R. similis* on Citrus

The experiments that were set up to determine whether or not solutions containing chlorine would affect the nematode populations in pots that contained citrus seedlings growing in naturally infested soil were dismantled after four months. Although wide variations among individual plants made differences statistically insignificant, the numbers of nematodes recovered from the treated plants differed sharply from the numbers collected from the check plants as shown in Table 3.

At the highest concentrations of chlorine (500 ppm), no nematodes were found. The plants had fewer and smaller leaves, with a tendency for yellowing as compared to the

Table 3. Measurements of sour orange seedlings, (1) untreated, (2) treated with calcium hypochlorite, (3) treated with sodium hypochlorite grown in naturally nematode infested soil and the numbers of _P. brachyurus_ and _R. similis_ recovered after four months.

Treatment	Height in inches	Fresh weight tops in grams	Fresh weight roots in grams	Numbers of _P. brachyurus_ and _R. similis_ recovered
Calcium hypochlorite 500 ppm	1. 8	2.1	3.2	0
	2. 6	1.0	0.8	0
	3. 9	3.5	2.7	0
	4. 8	2.7	2.4	0
	5. 7	2.3	1.8	0
Mean	7.6	2.3	2.2	0
200 ppm	1. 8	4.6	2.9	2
	2. 14	18.5	12.8	0
	3. 15	17.5	11.6	0
	4. 13	10.6	5.7	0
	5. 13	13.6	7.4	0
Mean	12.7	12.9	8.1	0.4
100 ppm	1. 10	11.6	7.4	0
	2. 19.5	21.4	13.3	0
	3. 10	8.6	5.3	2
	4. 12	11.7	8.4	3
	5. 12	11.8	8.7	1
Mean	12.7	13.0	8.6	1.2
50 ppm	1. 7	4.9	3.8	42
	2. 9	6.7	5.3	0
	3. 11	11.7	8.9	21
	4. 13	15.2	9.6	49
	5. 7	5.8	5.8	63
Mean	9.4	8.8	6.7	35
Sodium hypochlorite 100 ppm	1. 6	3.4	3.1	49
	2. 11	7.2	4.2	35
	3. 15	8.8	6.6	49
	4. 11	9.4	9.4	28
	5. 8	4.4	2.8	21
Mean	10.2	6.6	5.2	36.4
Checks	1. 10	8.0	8.6	532
	2. 11	9.4	7.2	427
	3. 16	13.8	10.2	479
	4. 13	12.8	11.4	413
	5. 14	12.4	9.7	441
Mean	12.8	11.3	9.4	458.4

leaves on seedlings receiving other treatments. With citrus plants, soil pH directly influences various nutrients necessary for plant growth. High contents of calcium in soil makes magnesium and potassium less available to citrus plants (20), and all the micronutrient elements except molybdenum are only slightly available at high pH values. It is likely that, at the high treatment rates, calcium accumulated in sufficient quantities to raise the soil to these higher pH values, thus making these nutrients unavailable to the plants.

At the 200 ppm and 100 ppm chlorine levels, the mean fresh weights of plant tops were higher than from the plants having no treatment and the total numbers of nematodes recovered from these plants were two and six, respectively. At the lowest level of chlorine (50 ppm), the nematodes recovered numbered an average of 35 per plant; whereas, the plants that were growing in untreated soil yielded an average of 458 nematodes per plant.

The plants that were treated with sodium hypochlorite solution were stunted, had less top and root weight than the untreated plants and the leaves were chlorotic. An average of 36 nematodes per plant was recovered.

The numbers of nematodes recovered from plants treated with chlorine solutions indicate nematocidal properties for these solutions. It would be interesting to compare results from several different chlorine-containing compounds as to their phytotoxicity and nematocidal properties, as well as their effect on the soil itself.

CONCLUSIONS

1. Members of the nematode genus *Pratylenchus* commonly occur in Florida citrus groves.

2. Certain populations of these *Pratylenchus* were studied in detail and found conspecific with *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Stekhoven, 1941. Other species were not identified.

3. *Pratylenchus brachyurus* will feed upon and reproduce within the roots of sour orange (*Citrus aurantium* L.) seedlings.

4. *Pratylenchus brachyurus* is pathogenic to sour orange; therefore, it may be suspected that other types of citrus are attacked and this nematode may be a factor in reducing yields in Florida.

5. The damage caused by *Pratylenchus brachyurus* is not so severe as that brought about by the nematode *Radopholus similis*. Thus, the former produces a disease which differs from spreading decline of citrus.

6. Calcium hypochlorite, applied as a drench to citrus plants growing in nematode infested soil, significantly lowered numbers of *P. brachyurus* and *R. similis* recovered from these plants as compared to numbers recovered from plants that had no treatment.

SUMMARY

The species of *Pratylenchus* that is predominant in Florida citrus groves was identified as *Pratylenchus brachyurus* (Godfrey, 1929) Filipjev and Stekhoven, 1941. The males of *P. brachyurus* observed during the course of this study, contrary to published description, have phasmide that extend into the bursa to form a small rib.

Pathogenicity of *P. brachyurus* to citrus was established by inoculating sour orange (*Citrus aurantium* L.) seedlings growing in sterile soil. Reduction in plant growth was observed in every case when inoculated plants were compared with uninoculated plants. Reduction of both top and root growth was significant when compared to the check plants. Reduction of both top and root growth was much more pronounced on plants infected with *Radopholus similis* than with *P. brachyurus*. There appears to be a basic difference in host response to the feeding activities of *P. brachyurus* and *R. similis*. Although both nematodes enter and feed upon the root tissue, *R. similis* appears to cause much more damage to the plant than does *P. brachyurus*.

P. brachyurus infected citrus roots were sectioned and stained. Destruction of parenchyma cells in the cortical region resulted in the formation of cavities where nematodes were found. The vascular system did not appear to be disturbed. Sections of roots infected by *R. similis* showed almost complete destruction in some cases. Nematodes were observed in the cortical region and the pericycle. Many more *R. similis* were present in lesions than were *P. brachyurus*.

P. brachyurus and *R. similis* infected citrus plants that were treated with calcium hypochlorite and sodium hypochlorite solutions as a soil drench yielded significantly lower numbers of nematodes than did plants that were not treated.

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BIOGRAPHICAL SKETCH

Troy Luther Brooks was born November 21, 1923, at Danielsville, Georgia. In June, 1941, he was graduated from Madison County High School. From 1942 through 1945 he served in the Air Corps of the United States Navy and was stationed in the Pacific. Following his discharge from the Navy, he entered the University of Georgia where he received the degree of Bachelor of Science in Agriculture in June, 1951. From July, 1951, until September, 1960, he worked as Assistant in Pathology at the University of Florida Citrus Experiment Station in Lake Alfred, Florida. In September, 1960, he enrolled in the Graduate School of the University of Florida where he received the degree of Master of Science in June, 1962. Until the present time he has pursued his work toward the degree of Doctor of Philosophy with a major in Entomology (Nematology).

Troy Luther Brooks is married to the former Robbie Ray Broome and is the father of two children. He is a member of the Society of Nematologists, Florida State Horticultural Society, Soil and Crop Science Society of Florida, American Phytopathological Society and Alpha Zeta.

This dissertation was prepared under the direction of the chairman of the candidate's supervisory committee and has been approved by all members of that committee. It was submitted to the Dean of the College of Agriculture and to the Graduate Council, and was approved as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

April 18, 1964

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